55. [New] An expression vector comprising a promoter sequence, a polypeptide encoding sequence, and a nucleic acid sequence of SEQ ID NO: 7 incorporated downstream of the promoter sequence and upstream of the polypeptide encoding sequence, wherein the nucleic acid sequence of SEQ ID NO: 7 enhances expression of the polypeptide by means of increasing IRES activity.

56. [New] The expression vector according to claim 55, wherein said gene expression vector is a vector for expression in eukaryotic cells.

II. REMARKS

A. SUBJECT MATTER OF THE CLAIMS

Generally, the subject matter of the amended claims is related to a 5'-untranslated region of a herpes simplex virus (HCV) viral gene used for enhancing expression of a useful gene in a gene expression construct by promoting translation activity in an IRES-dependent manner, expression constructs comprising an upstream promoter, the 5'-untranslated region of HCV, and a useful gene, host cells transformed or transfected with these constructs, methods for enhancing gene expression, and methods of using such molecules. Exemplary polynucleotide sequences of the 5'-untranslated region comprise: a nucleic acid sequence of either nucleotides 181-341 of SEQ ID NO: 1, having one thymidine inserted into position 207, or a fragment or variant thereof; or the nucleotide sequence of SEQ ID NO: 7, or a fragment or variant thereof.

Appendix A appended hereto is a marked-up version of the changes made to the claims by the present amendment and is captioned **"Versions with markings to show changes made."** Upon entry of the foregoing amendment, the pending claims in the application will appear as set forth in Appendix B appended hereto and captioned **"Pending claims upon entry of the present amendment."**

Support for new claims 47-56 is found throughout the original specification (including the claims) as originally filed.

B. OBJECTION TO PRIORITY AND DRAWINGS

At page 3 of the office action the examiner stated that an application in which the benefit of an earlier application is desired must contain a specific reference to the prior applications in the first sentence of the specification. The present application is a 35 U.S.C. § 371 national phase filing of a PCT application, which in turns claims priority from a Japanese

application under 35 U.S.C. § 119. It is the applicants' understanding that no specific cross-reference to a foreign 35 U.S.C. § 119 priority application should be made in the specification per se, in contrast to domestic priority claims. (The applicants will file a new inventor's declaration to clarify that no priority under 35 U.S.C. § 120 is claimed.)

The examiner also noted that there were drawing objections. The applicants will correct the drawings no later than payment of the issue fee.

C. PATENTABILITY REMARKS

1. Rejection of Claims 30, 32, 35, 36, and 46 Under 35 U.S.C. § 112, first paragraph, Based on Enablement Should be Withdrawn

On pages 3 through 8 of the official action, the examiner rejected claims 30, 32, 35, 36, and 46 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make or use the invention. Specifically, the examiner first asserted claims 30, 32, 35, 36, and 46 were drawn to broad methods of expressing and/or enhancing the expression of polynucleotide/therapeutic compositions for use in gene therapy to treat diseases resulting in the reduction of cap-dependent mRNA translation or a reduction in IRES activity. The examiner further alleged the specification is not enabling for use of these nucleic acid sequences in gene therapy and does not disclose any pharmaceutical property for the polynucleotides in the form of a composition. The examiner points out on page 5 of the official action that the prophetic examples (page 51, lines 4-6, lines 14-16 of the specification) "do not disclose the phenotype or behavior of the said organism after administrating the vectors containing these sequences and/or the effect of such vectors in exhibiting a therapeutic effect or mode of using the said polynucleotides [to treat] any disease in any organism." The examiner further alleges the specification does not provide sufficient guidance to practice the invention as claimed. In addition, the examiner stated that Verma *et al., Nature* 389:239 (1987), Marshall, *Science* 269:1054 (1995), and Orkin *et al.* allegedly teach that the gene therapy technology at the filing date of the invention was unpredictable. For example, at the time of publication of the above-identified publications, the examiner concluded that the delivery systems of therapeutic genes and the transient expression of these genes of interest were applied as a trial and error basis due to the availability of only poor vector expression systems. Therefore, the examiner concludes, the art was recognized as unpredictable for achieving therapeutic levels of gene expression and the specification

provided few parameters for affecting delivery and expression of therapeutic amounts of DNA into cells of interest for treating diseases.

The applicants respectfully traverse. Claims 30 and 32 are directed to a method of expressing and/or enhancing expression of a useful gene product using a vector comprising the nucleic acid of thymidine inserted at position 207 of SEQ ID NO: 1 or SEQ ID NO: 7 (no insert) for enhancing expression of a useful gene. One of skill in the art would generate a vector (using well known methodologies) with the applicants' novel sequences and a second gene of interest. Upon generation of the vector and administration of the vector constructs, one of skill in the art simply determines whether up-regulation of the gene product (from the gene of interest) has occurred via numerous well-known molecular-biology techniques such as immunochemistry.

The specification teaches generation of the vector for such as purpose. Specifically, example 8, page 44, line 17 to page 45, line 27 describe construction of a expression vector containing the unique polynucleotide sequence set out in SEQ ID NO: 7, a T7 promoter linked to a gene (R luc (luciferase)), and a T7 terminator. A second vector was constructed in a similar manner, but instead of SEQ ID NO: 7 as the gene enhancing element, the polynucleotide sequence set out in SEQ ID NO: 1 with an insertion of thymidine at position 207, was cloned in a similar manner generating the vector pT7-RL-UTR_{342A}. These enhancing elements may increase IRES activity. [See page 47, line 29 to page 48, line 23.] *In vitro* translation experiments showed that expression of luciferase was four times higher with both the vector (pT7-RL-UTR_{342}) containing the enhancer element SEQ ID NO: 7 and the vector pT7-RL-UTR_{342A} containing the enhancing element SEQ ID NO: 1 with the thymidine inserted into position 207. [*See* page 44, line 15 to line 25). *In vivo* experiments, wherein HEP G2 cells were transfected, showed that vectors pT7-RL-UTR342 (SEQ ID NO: 7) and pT7-RL-UTR342A (thymidine^{207} insert of SEQ ID NO: 1) were capable of enhancing the level of luciferase expression "6-7 times higher" than vector constructs without the unique enhancing 5'UTR HCV elements (i.e., SEQ ID NO: 7; $thymidine_{207}$ insert of SEQ ID NO: 1). [See page 46, lines 1-11.]

Dual luciferase constructs (bi-cistronic vectors i.e., pGL3R-UTR_{342} and pGL3R-UTR_{342A}) also showed enhanced expression when the translation enhancing elements from SEQ ID NO: 7 (in vector pGL3R-UTR_{342}) and the $thymidine_{207}$ insert of SEQ ID NO: 1 (pGL3R-UTR_{342A}) were used. [See, Example 11, page 46, line 15-26; figure 12.] These unique expression elements were not limited to a particular host cell or cell line. [See page

47, lines 22-28]. Therefore, the specification fully enables one of skill in the art to clone the novel vector constructs of claims 30 and 32 with its novel sequences (SEQ ID NO: 7; SEQ ID NO: 1 thymidine^{207}) that accelerate IRES activity and result in enhancing expression of a useful gene.

Moreover, the rejection is improper because the examiner has failed to identify any aspect of the claim that is not enabled. The examiner suggests that a therapeutic result is required to enable the claims, but the claims in fact recite protein expression and are successfully practiced even in the absence of a therapeutic result. The number of issued patents with claims of this type is legion. If the examiner is aware of legal authority requiring proof of successful therapeutic gene therapy to satisfy the enablement requirement for a method that recites simple protein expression, then the applicants request that it be identified in the next office action.

The rejection of claims 35 and 36 are now moot in light of the amendments to these claims. Amended claims 35 and 36 have been amended to simply recite a composition with the novel sequences (SEQ ID NO: 7; SEQ ID NO: 1 thymidine^{207}) for enhancing expression of a useful gene. Again, no end results (*i.e* cure of a disease) are required, it is simply a composition comprising the novel sequences (SEQ ID NO: 7; SEQ ID NO: 1 thymidine^{207}) for enhancing expression of a useful gene product. As discussed above, the specification provides amply guidance to construct such a composition using the polynucleotide sequences of the instant invention and one of skill in the art would be able to practice the claimed invention. In light of the foregoing amendments, the rejection of pending claims 35 and 36 under 35 U.S.C. § 112, first and second paragraph, should be withdrawn. Furthermore, the applicants do not intend by these or any other amendments to abandon the subject matter of the claims as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

Finally, the applicants submit that the rejection of claim 46 based upon 35 U.S.C. § 112, first paragraph, is now moot. Specifically, and solely for the purpose of expediting prosecution, and without prejudice to the applicants' right to seek broader claims in a continuing application, the applicants have cancelled claim 46 without prejudice. Therefore, the applicants respectfully request that the rejection of claims 30, 32, 35, 36, and 46 under 35 U.S.C. § 112, first paragraph, as lacking enablement be withdrawn.

2. Rejection of Claims 37 and 43 Under 35 U.S.C. § 112, first paragraph, Based on Enablement Should be Withdrawn

On pages 8 through 11, the examiner outlined his rejection of claims 37 and 43 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make or use the invention. Specifically, the examiner first asserted claims 37 and 43 were drawn to methods for determining the severity of HCV infection in any and all subjects by detecting the presence of any and all variants of the HCV 5' UTR using the nucleic acid sequence of SEQ ID NO: 7. The examiner alleged that the specification is not enabling for other variants of HCV 5'UTR other than the nucleic acid HCV 5'UTR sequences of SEQ ID NO: 1 and SEQ ID NO: 7. The examiner points out that Borman *et al.* (1995) teaches the functional requirements for efficient IRES activity can vary dramatically depending upon a particular sequence. Therefore, the level of severity to a HCV infection becomes an unpredictable issue when corresponding it to a level of enhancement exhibited by SEQ ID NO: 7 or any other HCV UTR. Furthermore, the examiner alleges Laporte *et al. J. of Virol.* 74:10827-10833 (2000) states that HCV has a very high mutation rate and circulates as a population of closely related genomes. Finally, the examiner alleges that the "association between SEQ ID NO: 7 which is just one variant of SEQ ID NO: 1 of HCV1b and hyperviremia is always not definitive and the hyperviremia exhibited by the patient carrying SEQ ID NO: 7 could be due to any other variant present in the patient's system." Therefore, the examiner concludes that the lack of guidance regarding the types of mutations and the associated severity of infection would required undue experimentation on the part of a skilled artisan to practice the invention as recited in claims 37 and 43.

The applicants respectfully traverse the examiner's rejection of claims 37 and 43 in view of the fact that because one of skill in the art would have little difficulty in determining the presence of a particular nucleotide sequence such as SEQ ID NO: 7 in a particular biological sample. Likewise, the application enables one of skill in the art to correlate the presence of SEQ ID NO: 7 to a patient suffering from hepatitis C hyperviremia. SEQ ID NO: 7, whose sequence is derived from a variant strain of hepatitis C, was isolated from a patient who was suffering from hepatitis C hyperviremia. The examiner's argument that the "hyperviremia exhibited by the patient carrying SEQ ID NO: 7 **could be due** to any other variant present in the patient's system" is entirely without factual basis. In contrast, the

translational efficiency experiments outlined in the specification, however, showed that this unique 5' UTR (SEQ ID NO: 7) of a HCV variant enhances translational efficiency of unrelated genes, such as the Renilla luciferase (Rluc) gene. [See Examples 9-12, pages 43-47.] Therefore, one of skill in the art would believe that the presence of SEQ ID NO: 7 also enhances the translational efficiency of hepatitis C genes, which leads to a faster generation time of new progeny hepatitis C particles, which leads to an increase number of cells being infected at a faster rate, thereby leading to hyperviremia. Thus, the SEQ ID NO: 7 can be linked to the severity of a hepatitis C strain. [See page 48, lines 24-28.] Thus, the applicants respectfully request the rejection of pending claim 37 under 35 U.S.C. § 112, first paragraph, be withdrawn.

3. Rejection of Claims 1-6, 8-18, 20-25, 38-40, 44, and 45 Under 35 U.S.C. § 112, second paragraph, Based Being Indefinite For Failing to Particularly Point Out and Distinctly Claim the Subject Matter Should be Withdrawn

On pages 11 and 12 of the official action, the examiner rejected claims 1-6, 8-18, 20-25, 26, 32, 33, 38-40, 41, 44, and 45 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The applicants traverse in part and have amended the claims in part.

With respect to claims 33 and 41, the examiner alleges these claims are indefinite in their recitation of the word "substances." The applicants respectfully traverses.

The specification defines "substances that interact with IRES" on page 14, lines 12 to 18. The applicants therefore submit that the assertedly indefinite "substances" term in pending claim 33 is fully defined in the specification and the rejection under 35 U.S.C. § 112, second paragraph should be withdrawn.

With respect to the rest of the other claims, the applicants have amended these claims and the rejections under 35 U.S.C. § 112, second paragraph, are now moot.

4. Rejection of Claims 1-6, 8-13, 16, 21, 22, 28, 29, 31, 32, 35, 44, and 45 Under 35 U.S.C. § 102(b) Should be Withdrawn

On pages 13 and 14 of the official action, the examiner rejected claims 1-6, 8-13, 16, 21, 22, 28, 29, 31, 32, 35, 44, and 45 under 35 U.S.C. § 102(b) as allegedly being anticipated by Yoo *et al* (1992). The examiner asserted that Yoo *et al.* (1992) teaches a vector comprising a nucleic acid sequence of the 5' untranslated region (UTR) of hepatitis C virus

(HCV) enhancing the expression CAT protein *in vitro* and CAT enzymatic activity *in vivo* in mammalian cells. More specifically, the examiner asserts several deletion constructs of nucleotides 1-341 of the 5'UTR were used to study expression-enhancing ability of HCV 5'UTR. With regards to the 5'UTR of HCV, the examiner alleges the presence of a pyrimidine tract, a transfactor binding site or a ribosome landing pad, AUG or ATG codons within the open reading frames (ORFs), and an internal ribosome entry site (IRES) are all inherent features of the 5' UTR of HCV. Furthermore, the examiner states Yoo *et al.* (1992) teaches the 5' UTR region and therefore teaches the inherent feature of the 5' UTR sequence of HCV. The examiner also alleges because Yoo *et al.* (1992) teaches the sequence, it also teaches fragments or variants thereof as stated in claim 21. Finally, the examiner argues claim 35 is anticipated because the claim is directed to a therapeutic composition, which is the nucleic acid sequence inherently taught by Yoo *et al* (1992).

With respect to claims 1-6, 8-13, and 16, the applicants submit that the rejection based upon 35 U.S.C. § 102(b) in light of Yoo *et al* (1992) is now moot.

Specifically, and solely for purpose of expediting prosecution and without prejudice to the applicants' right to seek broader claims in a continuing application, the applicants have amended claims 21, 22, 28, 29, 31, 32, 35, 44, and 45 to distinguish their claimed invention from the disclosure of Yoo *et al* (1992).

Amended claims 21, 22, 28, 29, 31, 32, 35, 44, and 45 are directed to two novel sequences of the 5' UTR region of HCV. Claim 21 is directed to a nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted into position 207 (i.e., inserted between 206 and 207 of SEQ ID NO: 1). The consequence of this insertion at position 207 is that the claimed sequence as a whole is different from the "inherent" sequence taught in Yoo *et al* (1992) by at least this thymidine. The insertion of thymidine at position 207 of SEQ ID NO: 1 as taught by the applicants provides up to 6-7 times higher luciferase expression activity over HCV_{341} or the sequence taught in Yoo *et al* (1992). [See page 44, lines 15-21; page 46, lines 1-11 in light of page 43, lines 9-16.] The second novel sequence (SEQ ID NO: 7) is directed to claim 24, which is directed to the HCV_{342} isolate that comprises 342 nucleotides of the 5' UTR of HCV with a thymidine at position 207 along with a unique sequence directed to nucleotides 208-342 from the "inherent" sequence taught in Yoo *et al* (1992). SEQ ID NO: 7 also provides up to 6-7 times higher luciferase expression than the same sequence (HCV341) taught in Yoo *et al* (1992) [See page 44, lines 15-25; page 46, lines 1-6.]

All of the other rejected claims depend from either claim 21 or 24 or contain analogous limitations that are distinguishable over Yoo *et al.* (1992). Therefore, the applicants respectfully request the rejection under 35 U.S.C. § 102(b) in view of Yoo *et al.* (1992) should be withdrawn.

5. Rejection of Claims 23-25, 27, 41, 42, and 46 Under 35 U.S.C. §102(b) Should be Withdrawn

On page 15 and the top of page 16 of the official action, the examiner also rejected claims 23-25, 27, 41, 42, and 46 as being anticipated by Collier *et al.* (1998). The examiner alleges that Collier *et al* (1998) teaches "a novel bicistronic dual luciferase reporter construct assay system for studying translational efficiencies of 5' UTR from hepatitis C virus" wherein the luciferase translation is directed by the IRES element within the 5' UTR element of hepatitis C. As with the examiner's rejection of claim 35 above, the examiner rejected claim 46 because this claim is directed to a claimed composition that is anticipated by Collier *et al.* (1998).

Specifically, and solely for purposes of expediting prosecution and without prejudice to the applicants' right to pursue claims in a continuing application, the applicants have cancelled claims 25, 27, 41, 42, and 46. Accordingly, the rejection is moot for these claims.

Amended claims 23 and 24 are directed to two novel sequences of the 5'UTR region of HCV. As discussed above, claim 23 depends from claim 21, which is directed to a novel nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted into position 207 of SEQ ID NO: 1. None of the HCV 5'UTR sequences disclosed in Collier *et al.*, contain this thymidine in position 207 of SEQ ID NO: 1 and even though Collier *et al.* teaches a dual luciferase reporting construct assay system, Collier *et al.*, does not teach the 5'UTR sequence with a thymidine inserted into position 207 of SEQ ID NO: 1. Collier *et al.*, similarly fails to teach the 5'UTR sequence of SEQ ID NO: 7 as recited in claim 24. Both of these claims recite a unique sequence with both a thymidine insertion at position 207 (and cytosine substitution at position 119 of SEQ ID NO: 7) in comparison to the seven HCV 5'UTR sequences taught in Collier *et al.* Similarly, claim 46 is directed to a unique composition comprising nucleic acid sequence of claim 24 and is therefore not anticipated by Collier *et al.* Thus, Collier *et al.* fails to teach every element set forth in claims 23 and 24 and therefore the rejection under 35 U.S.C. § 102(b) may be withdrawn.

6. Rejection of Claims 33 and 34 Under 35 U.S.C. § 102(b) Should be Withdrawn

The examiner rejected claims 33 and 34 under 35 U.S.C. § 102(b) as allegedly being anticipated by Zhang (1998). The examiner asserted that Zhang (1998) teaches the polynucleotide of SEQ ID NO: 7 as part of the sequence of the GenBank Accession # AB016785. Examiner further alleges that since claims 33 and 35 are directed to a probe comprising the polynucleotide of SEQ ID NO: 7, "the intended use of the claimed probe is given patentable weight when making a determination of patentability under 35 U.S.C. 102 when it serves to define a structural requirement." Therefore, the examiner concludes in view of the comprising language, " the prior art structure has all the features required to perform the intended use recited in the claims."

Claims 33 and 34 are not anticipated by Zhang *et al.* As the transmittal papers in Appendix C show, the above-identified application is a U.S. national phase of the Japanese PCT application number PCT/JP99/03682, which has an international filing date of July 7, 1999. This PCT filing date is within a year of the publication date of Zhang (August 5, 1998) and therefore Zhang *et al.* cannot properly anticipate claims 33 and 35 under 35 U.S.C. § 102(b) (See 35 U.S.C. § 363).

7. Rejection of Claims 23-25, 27, 41, 42, and 46 Under 35 U.S.C. § 103 Should be Withdrawn

On page 17 and 18 of the office action, the examiner rejected claims 23-25, 27, 41, 42, and 46 under 35 U.S.C. § 103(a) for being directed to subject matter allegedly rendered obvious in light of the disclosure of Brown *et al., Nucleic Acid Research* 20:5041-5045 (1992) (hereafter Brown et al. (1992)), Dirks *et al., Gene* 128:247-249 (1993) (hereafter Dirks *et al.* (1993)) in view of Fukushi *et al., Journal of Virology* 71:1662-1666 (1997). Applicants respectfully traverse.

Specifically, the examiner alleges Brown *et al.* (1992) teaches the sequence and the structure of the 5' nontranslated region of hepatitis C virus but does not teach the 5' nontranslated region of HCV as an IRES. The examiner further alleges Dirks *et al.* (1993) teaches using dicistronic vectors that utilize the IRES sequences of poliovirus for gene expression in mammalian cells, but does not teach using this method and vector construct with the HCV 5' UTR sequences. Finally, the examiner states Fukushi *et al* (1997) teaches a translation strategy used by HCV that is similar to the strategy employed by members of the

family Picornavidae includes similar IRES regions. Therefore, the examiner concludes Fukushi *et al.* provides the motivation to use HCV 5'UTR as an IRES wherein one of ordinary skill in the art could substitute the poliovirus IRES with the HCV 5' UTR IRES in the vector of Dirks *et al.* (1993).

To establish a *prima facie* case of obviousness, at least three basic criteria must be met. First, the prior art must teach or suggest all of the claim limitations. Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Finally, there must be a reasonable expectation of success. [*In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).]

A. The cited references do not teach or suggest all of applicants' claimed limitations

The primary references, Brown *et al.* (1992) and Dirk *et al.* (1993), either alone or in combination with each other or the secondary reference Fukushi *et al.* neither teach nor suggest the required elements of the Applicants claimed invention. As a preliminary matter, the applicants submit that the rejection of claims 25, 27, 41, 42, and 46 are now moot in that the applicants have canceled these claims (without prejudice to the applicants' right to seek broader claims in a continuing application).

Claims 23 and 24 are directed to enhancing useful gene expression by utilizing the IRES activity of the 5' UTR region with the specific requirement that the unique thymidine at position 207 of the 5' untranslated region of HCV be present. While Brown *et al.* (1993) simply teaches the sequence of the 5' nontranslated region of HCV and its secondary structure and compares the secondary structure to other 5' nontranslated regions of the pestiviruses, bovine viral diarrhea virus (BVDV), and the hog cholera (HChV) virus, Brown *et al.* (1993) does not teach or suggest the unique thymidine at position 207 as recited in claim 23 and 24. This thymidine at position 207 of the 5' untranslated region of HCV enhances expression of useful genes at a higher level than without the thymidine insertion at position 207 of the 5' UTR of HCV, a result that could not have been predicted from the prior art. With respect to Dirks *et al.*, the cited reference is nothing more that a general reference for a vector setup. As with Brown *et al.*, Dirks *et al.* does not teach nor suggest the thymidine at position 207 as required by the applicants' claimed invention

The secondary reference, Fukushi *et al.*, does nothing to rehabilitate the deficiencies of Brown *et al.* or Dirks *et al.* Rather, Fukushi *et al.* merely discloses the identification of a novel 25-kDa protein that specifically interacts with the HCV IRES region identified by mutational studies of the alpha-branch structure (nucleotides 47-67) of the wild type HCV UTR_{341} region. As discussed above, Fukushi *et al.* neither teaches or suggests the unique thymidine at position 207 of SEQ ID NO: 1 and/or the unique thymidine or cysteine in SEQ ID NO: 7. Therefore, the above cited references fail to teach or suggest any of the HCV 5' UTR sequences specific for claims 23 and 24.

B. The cited references do not motivate one of skill in the art to modify the referenced teachings.

The second prong for establishing a *prima facie* case of obviousness also fails. On page 18 of the official action, the examiner alleges Fukushi *et al.* teaches the HCV IRES is similar to that of picornaviruses and thereby provides the motivation to use the 5' UTR as an IRES. Applicants respectfully disagree.

As discussed above, Fukushi *et al.* teaches that mutation studies of the cis-acting alpha-branch (nucleotides 47-67) of the wild type HCV UTR_{341} region indicated that this cis-acting element was critical for translational initiation and identified a particular 35-kDa protein that interacted with this specific region. Based upon this teaching, nucleotides 47-67 of HCV 5'UTR are **critical for translational initiation**, but provide no insight into other critical nucleotides of the 5' UTR such as thymidine 207. As taught by applicants' disclosure, thymidine 207 **provides upregulation of translation promoting activity, which enhances expression of useful genes**. Therefore, there is no motivation to modify Brown *et al.* and Dirks *et al.* in light of Fukushi *et al.*, because Fukushi *et al.* simply identifies the region for initiation of translation process, but does not identify the modification at position 207 of the 5'UTR of HCV, which enhances the efficiency of translation. Therefore, the above cited references fail to provide a motivation to one of skill in the art to modify the above cited references in accordance with pending claims 23 and 24.

C. Unexpected Results

Notwithstanding the above, the applicants are directed to a set of isolated polynucleotides with the unexpected property of enhancing expression of useful genes (*in vitro* and *in vivo*) and provided an activity up to 6-7 times higher than the activity of a vector

with the wild type HCV 5'UTR$_{341}$ sequence. Specifically, the presence of thymidine in position 207 of the HCV-derived sequences (insertion into SEQ ID NO: 1 and present in SEQ ID NO: 7) increases the effect of enhancing the expression of useful genes in a cell-nonspecific manner. [See Example 9, page 44, lines 15-25; Example 10, page 46, lines 1-6; Example 12, page 47, lines 11-28.] Brown *et al.* in figure 2, page 5043 teaches the sequence of the 5'UTR of HCV but not the thymidine at position 207. Dirks *et al.* simply teaches dicistronic vectors utilizing IRES sequences of polioviruses. Thus, Brown *et al.* or Dirks *et al.* fail to exhibit the increased enhancing activity of thymidine 207. Therefore, the applicants' invention has unexpected properties that are not obvious in light of the above-cited references.

D. Summary

The applicants submit that the disclosures of Brown *et al.* (1992) and Dirks *et al.* (1993) in view of Fukushi *et al.* (1997) cannot render obvious any of the presently claimed subject matter due to a failure to teach all of the claim limitations, or provide motivation to combine the references. In addition, the applicants' invention has the unexpected property of increasing the effect of enhancing the expression of useful genes in a cell-nonspecific manner. Therefore, the applicants submit the rejection under 35 U.S.C. § 103 should be withdrawn.

III. CONCLUSION

The foregoing amendments and remarks are believed to establish that claims 21-24, 26, 28-40, 44, 45, and 47-55 are in condition for allowance, and an early notice thereof is respectfully solicited.

Respectfully submitted,

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October 10, 2002

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

Please cancel claims 1-18 and 20 without prejudice.

Please amend claims 21-24 as follows.

21. [Twice Amended] [A nucleic acid sequence] An isolated polynucleotide for enhancing protein expression, said polynucleotide [of a useful gene incorporated into a gene expression vector for enhancing expression of a useful gene] comprising a nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted [into position 207] between positions 206 and 207 of SEQ ID NO: 1, or a fragment [or variant]thereof that includes said thymidine, wherein said polynucleotide or fragment enhances protein expression when incorporated downstream of an expression regulatory promoter sequence and upstream of a protein coding sequence.

22. [Twice Amended] [The nucleic acid sequence for enhancing expression of a useful gene] The isolated polynucleotide according to [claim 1 or] claim 21, wherein said nucleic acid sequence has translation promoting activity to [enhancing] enhance expression of a nucleic acid sequence encoding a protein sequence [of a useful gene enhances expression of a useful gene by means of its own translation promoting activity].

23. [Twice Amended] [The nucleic acid sequence for enhancing expression of a useful gene] The isolated polynucleotide according to [claim 1 or] claim 21, wherein said nucleic acid sequence [for enhancing expression of a useful gene] enhances said expression [of a nucleic acid sequence] by [means of accelerating] increasing IRES activity.

24. [Twice Amended] [A nucleic acid sequence for enhancing expression of a useful gene] An isolated polynucleotide that enhances protein expression when included 5' of a protein coding sequence in an expression construct by promoting mRNA translation in an IRES dependent manner, said polynucleotide comprising a nucleotide sequence of SEQ ID NO: 7. [, which enhances expression of a nucleic acid sequence by means of promoting mRNA translation in an IRES-dependent manner.]

Please cancel claim 25 without prejudice.

Please amend claim 26 as follows.

26. [Twice Amended] An isolated polynucleotide consisting of [a] the nucleotide sequence [of] as set forth in SEQ ID NO: 7 over its entire length.

Please cancel claim 27 without prejudice.

Please amend claims 28, 30, and 31 as follows.

28. [Twice Amended] [A gene] An expression vector comprising [the nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 1 or claim 21] an isolated polynucleotide according to claim 21 or claim 24.

30. [Amended] A method of expressing a [useful gene product using the according to claim 28] protein comprising the steps of:

(a) transforming or transfecting a host cell with an expression vector according to claim 53,

(b) growing the host cell in a medium under conditions where the cell expresses the protein.

31. [Amended] A method [for producing a useful gene product comprising the steps of: growing the host cell according to claim 29 in a medium; and isolating the useful gene product] according to claim 30, further comprising a step of isolating the protein from the cell and/or the growth medium.

Please cancel claim 32, without prejudice.

Please amend claims 33 -39 as follows

33. [Amended] A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26, further comprising a detectable label.

34. [Amended] A probe for screening IRES-dependent translation inhibitors, comprising the polynucleotide according to claim 26, further comprising a detectable label.

35. [Twice Amended] A [therapeutic] composition [for treating diseases resulting from reduction of cap-dependent mRNA translation in a body of organism,] comprising the [nucleic acid sequence] isolated polynucleotide for enhancing protein expression according to claim 21. [of a useful gene according to claim 1 or claim 21 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms].

36. [Amended] A [therapeutic] composition [for treating diseases resulting from reduction of cap-dependent mRNA translation in a body of organism,] comprising the [nucleic acid sequence] isolated polynucleotide for enhancing protein expression according to claim 24. [of a nucleic acid sequence according to claim 24 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.]

37. [Twice Amended] A method for determining [the severity of] a hypervirulent hepatitis C strain, comprising the steps of: [detecting the presence of a target polynucleotide sequence contained in a biological sample derived from a test subject, by using the polynucleotide according to claim 26 or claim 27 as the target; and determining the presence of hepatitis C based on the presence of the sequence.]

(a) screening a biological sample for the presence of the polynucleotide according to claim 26, and;

(b) determining presence or absence of the hypervirulent hepatitis C strain from the screening step, wherein the presence of the polynucleotide identifies the hypervirulent hepatitis C strain in the biological sample and the absence of said sequence indicates the absence of said hypervirulent hepatitis C.

38. [Twice Amended] [The nucleic acid sequence] An isolated polynucleotide [for enhancing expression of a nucleic acid sequence] according to claim 21, further comprising [a nucleic acid sequence of] nucleotides 1-180 of SEQ ID NO: 1. [having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof.]

39. [Twice Amended] [The nucleic acid sequence] An isolated polynucleotide [for enhancing expression of a nucleic acid sequence] according to claim 21 or 38, further comprising [a nucleic acid of] nucleotides 342-713 of SEQ ID NO: 1. [having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof].

Please cancel claim 40-43, without prejudice.

Please amend claim 44 and 45 as follows.

44. [Twice Amended] [The nucleic acid sequence] An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to [claim 1 or] claim 24, wherein the 5'-untranslated region comprises a polynucleotide sequence corresponding to at least one region selected from the group

consisting of pyrimidine-rich tract, Box A, Box B, a trans factor-binding site, and a combination thereof.

45. [Twice Amended] [The nucleic acid sequence] An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 44, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion, and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or proximate sequence in at least one position corresponding to a pyrimidine-rich tract, Box A, Box B and/or trans factor-binding site contained in the 5'-untranslated region.

Please cancel claim 46, without prejudice.

Please add new claims 47-56.

47. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

48. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of Box A, Box B, a trans-binding site, and a combination thereof.

49. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises an AUG or ATG sequence.

50. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a part of or an entire region of IRES of viral mRNA.

51. [New] The isolated polynucleotide according to claim 24, wherein said nucleic acid further comprises a portion of a coding region from a viral gene adjacent to the 5'-untranslated region.

52. [New] The isolated polynucleotide according to claim 24, wherein said nucleic acid is a cDNA sequence.

53. [New] An expression vector according to claim 28, further comprising a protein coding sequence operably inserted downstream of the polynucleotide for enhancing protein expression.

54. [New] An isolated polynucleotide comprising nucleotide 181-341 of SEQ ID NO: 1, wherein said polynucleotide includes a thymidine inserted between position 206 and 207 of SEQ ID NO: 1.

55. [New] An expression vector comprising a promoter sequence, a polypeptide encoding sequence, and a nucleic acid sequence of SEQ ID NO: 7 incorporated downstream of the promoter sequence and upstream of the polypeptide encoding sequence, wherein the nucleic acid sequence of SEQ ID NO: 7 enhances expression of the polypeptide by means of increasing IRES activity.

56. [New] The expression vector according to claim 55, wherein said gene expression vector is a vector for expression in eukaryotic cells.